

Criteria Specification

ClinGen Cerebral Creatine Deficiency Syndromes Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for GAMT Version 1.1.0

Affiliation: Cerebral Creatine Deficiency Syndromes VCEP

Description : Cerebral Creatine Deficiency Syndromes Variant Curation Expert Panel ACMG Classification Rules Specified for GAMT (guanidinoacetate methyltransferase) Summary of ACMG-AMP Criteria for GAMT Variants

Version : 1.1.0

Released : 9/14/2022

Release Notes :

- Added supporting strength for PM3 and PM4. This was previously approved by SVI for Version 1.0 but had not been included in the CSpec Registry.
- Added clarification for points system for PP4, all previously approved by SVI.

Rules for GAMT

Gene: GAMT (HGNC:4136) [↗](#)

Preferred Transcript: NM_000156.6

HGNC Name: guanidinoacetate N-methyltransferase

Disease: guanidinoacetate methyltransferase deficiency (MONDO:0012999) [↗](#)

Criteria & Strength Specifications

PVS1

Original ACMG Summary

Null variant (nonsense, frameshift, canonical \pm 1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7).
- Use caution interpreting LOF variants at the extreme 3' end of a gene.
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.
- Use caution in the presence of multiple transcripts.

Very Strong

Nonsense-mediated decay predicted

CCDS VCEP notes:

Loss of function (LOF) of GAMT is a known mechanism of disease for guanidinoacetate methyltransferase deficiency (GAMT-D). There are examples of various LOF variants,

including nonsense and frameshift, in GAMT in individuals with GAMT-D (<https://databases.lovd.nl/shared/variants/GAMT/unique>). The specifications below are based on the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042).

Nonsense and frameshift variants

* All nonsense and frameshift variants will meet PVS1 unless a premature termination codon is predicted to be missed by nonsense-mediated decay (NMD) because it is located in the last exon (exon 6) or the last 50 bases of the penultimate exon of the gene (exon 5, c.520). In that case, PVS1_Strong or PVS1_Moderate will be applied, depending on whether >10% or <10% of the protein is lost.

Splice site variants (+1, +2, -1, -2)

* All canonical splice site pairs in GAMT are GT-AG.

* For any canonical splice site variant (+1, +2, -1, -2), the exon immediately adjacent to the variant is predicted to be skipped i.e. upstream exon skipped for canonical donor splice site variants and downstream exon skipped for canonical acceptor splice site variants.

* For the predicted in frame/out of frame consequences of exon skipping and assigned strength of PVS1, see [Appendix 1](#).

* If this criterion is applied, PP3 (in silico splice site prediction tools) should not be used.

* To apply PVS1, splice site variants must have no detectable nearby (+/- 20 nucleotides) strong consensus splice sequence that may reconstitute in-frame splicing. Otherwise, the PVS1 strength should be reduced accordingly.

* Non-canonical splice variants, such as +3 or -3, will not meet PVS1, but could meet PS3 and/or PP3 criteria.

Deletions (single or multi exon)

* If a single or multi-exon deletion results in an out of frame consequence, use PVS1 unless not predicted to undergo NMD. If not predicted to undergo NMD, use PVS1_Strong if >10% of the protein is predicted to be removed, and use PVS1_Moderate if <10% of the protein is predicted to be removed.

* If the consequence is in frame, the deletion must encompass one or more exons for PVS1 to apply. Use PVS1_Strong if more than 10% of the protein is removed and PVS1_Moderate if <10% of the protein is removed.

* If the in frame deletion is smaller than one exon, PVS1 does not apply; consider using PM4.

* [Appendix 1](#) can be used to predict the consequences of single exon deletions.

Duplications

* Use the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042) to assess the impact of duplications.

Modification None

Type:

Strong

In frame loss of >10% of the protein.

Loss of function (LOF) of GAMT is a known mechanism of disease for guanidinoacetate methyltransferase deficiency (GAMT-D). There are examples of various LOF variants, including nonsense and frameshift, in GAMT in individuals with GAMT-D (<https://databases.lovd.nl/shared/variants/GAMT/unique>). The specifications below are based on the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042).

Nonsense and frameshift variants

* All nonsense and frameshift variants will meet PVS1 unless a premature termination codon is predicted to be missed by nonsense-mediated decay (NMD) because it is located in the last exon (exon 6) or the last 50 bases of the penultimate exon of the gene (exon 5, c.520). In that case, PVS1_Strong will be applied if >10% of the protein is lost.

Splice site variants (+1, +2, -1, -2)

- * All canonical splice site pairs in GAMT are GT-AG.
- * For any canonical splice site variant (+1, +2, -1, -2), the exon immediately adjacent to the variant is predicted to be skipped i.e. upstream exon skipped for canonical donor splice site variants and downstream exon skipped for canonical acceptor splice site variants.
- * Use SpliceAI and varSEAK to look for nearby (+/- 20 nucleotides) strong consensus splice sequence that may reconstitute in-frame splicing.
- * For considerations for strength at which PVS1 may be applied see [Appendix 1](#).
- * If this criterion is applied, PP3 (in silico splice site prediction tools) should not be used.
- * Non-canonical splice variants, such as +3 or -3, will not meet PVS1, but could meet PS3 and/or PP3 criteria.

Deletions (single or multi exon)

- * If a single or multi-exon deletion results in an out of frame consequence, use PVS1 unless not predicted to undergo NMD. If not predicted to undergo NMD, use PVS1_Strong if >10% of the protein is predicted to be removed, and use PVS1_Moderate if <10% of the protein is predicted to be removed.
- * If the consequence is in frame, the deletion must encompass one or more exons for PVS1 to apply. Use PVS1_Strong if more than 10% of the protein is removed and PVS1_Moderate if <10% of the protein is removed.
- * If the in frame deletion is smaller than one exon, PVS1 does not apply; consider using PM4.
- * [Appendix 1](#) can be used to predict the consequences of single exon deletions.

Duplications

- * Use the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042) to assess the impact of duplications.

Modification Strength

Type:

Moderate

Single exon or larger deletion resulting in loss of <10% of the protein.

Initiator codon variant.

CCDS VCEP notes:

Loss of function (LOF) of GAMT is a known mechanism of disease for guanidinoacetate methyltransferase deficiency (GAMT-D). There are examples of various LOF variants, including nonsense and frameshift, in GAMT in individuals with GAMT-D (<https://databases.lovd.nl/shared/variants/GAMT/unique>). The specifications below are based on the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042).

Nonsense and frameshift variants

* All nonsense and frameshift variants will meet PVS1 unless a premature termination codon is predicted to be missed by nonsense-mediated decay (NMD) because it is located in the last exon (exon 6) or the last 50 bases of the penultimate exon of the gene (exon 5, c.520). In that case, PVS1_Moderate will be applied if <10% of the protein is lost.

Splice site variants (+1, +2, -1, -2)

* All canonical splice site pairs in GAMT are GT-AG.

* Use SpliceAI and varSEAK to look for nearby (+/- 20 nucleotides) strong consensus splice sequence that may reconstitute in-frame splicing.

* For any canonical splice site variant (+1, +2, -1, -2), if RT-PCR data predicts in-frame loss of <10% of the protein, apply PVS1_Moderate.

* If this criterion is applied, PP3 (in silico splice site prediction tools) should not be used.

* Non-canonical splice variants, such as +3 or -3, will not meet PVS1, but could meet PS3 and/or PP3 criteria.

Initiator codon variants

* To our knowledge, initiator codon variants have not been reported in GAMT (01/2019) but may occur.

* All initiator codon variants will meet PVS1_Moderate. The next in-frame methionine is at amino acid position 42 (based on NP_000147.1).

Deletions (single or multi exon)

* If a single or multi-exon deletion results in an out of frame consequence, use PVS1 unless not predicted to undergo NMD. If not predicted to undergo NMD, use PVS1_Strong if >10% of the protein is predicted to be removed, and use PVS1_Moderate if <10% of the protein is predicted to be removed.

* If the consequence is in frame, the deletion must encompass one or more exons for PVS1 to apply. Use PVS1_Strong if more than 10% of the protein is removed and PVS1_Moderate if <10% of the protein is removed.

* If the in frame deletion is smaller than one exon, PVS1 does not apply; consider using PM4.

* [Appendix 1](#) can be used to predict the consequences of single exon deletions.

Duplications

* Use the PVS1 decision tree (Figure 1, Abou Tayoun et al, 2018, PMID 30192042) to assess the impact of duplications.

Modification Strength

Type:

Original ACMG

Summary

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.

Example: Val->Leu caused by either G>C or G>T in the same codon.

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

This criterion is applicable as described.

If the variant is in the last 3 nucleotides of an exon, further analysis using splicing site prediction algorithms (see PP3) and data from the literature (if available) is required to investigate the impact on splicing.

Modification None

Type:

PS2

Original ACMG

Summary

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history.

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

Not Applicable

Comments:

De novo (both maternity and paternity confirmed) in a patient with the disease and no family history. Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to non-maternity.

PS3

Original ACMG

Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.

Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Strong

RT-PCR evidence of mis-splicing for non-canonical +1, +2, -2, -1 intronic variants with no evidence of normal splice products.

GAMT specifications:

Any variant meeting the description for splicing assays below can meet PS3 or PS3_Supporting, and variants meeting the description for in vitro activity assays can meet PS3_Supporting. If a variant meets the description for both e.g., a splice site variant with evidence of abnormal splicing and deficient GAMT activity in vitro, PS3 must only be counted once.

Splicing assays

For non-canonical splicing variants, use PS3 if there is RT-PCR and/or RNA sequencing evidence demonstrating only abnormal splice products, with no evidence of normal splicing.

* Evidence of abnormal splicing includes transcripts of alternative length or with specific intron or exon inclusion/exclusion. These studies can be performed on mRNA extracted from patient-derived cells, or by inserting the mutant genomic DNA into plasmid vectors and introducing these into human or other mammalian host cells.

* Note that in patients who are compound heterozygotes for a splicing variant and another variants type that does not disrupt splicing, such as a missense variant, evidence of normal splicing is expected. However, the presence of normal splice products could complicate the assessment of the impact of the splice variant. Therefore, if there is any evidence of normal splice products, either when using RNA from patient cells or in an in vitro expression system, use PS3_Supporting.

* PP3 may also be used for non-canonical splice variants meeting PS3 or PS3_Supporting.

Modification Disease-specific

Type:

Supporting

<15% control activity when variant is expressed in GAMT-deficient fibroblasts or HeLa cells.

*** RT-PCR evidence of mis-splicing for non-canonical intronic variants with evidence of normal splice products.**

GAMT specifications:

Any variant meeting the description for splicing assays below can meet PS3 or PS3_Supporting, and variants meeting the description for in vitro activity assays can meet PS3_Supporting. If a variant meets the description for both e.g., a splice site variant with evidence of abnormal splicing and deficient GAMT activity in vitro, PS3 must only be counted once.

In vitro expression

PS3_Supporting can be assigned if a variant is expressed in GAMT-deficient fibroblasts or HeLa cells and has <15% of the control value, for values published in Mercimek-Mahmutoglu et al, 2014, PMID 24415674; Mercimek-Mahmutoglu et al, 2016, PMID 26319512; or DesRoches et al, 2016, PMID 26003046. See [Appendix 2](#) for further details on GAMT functional assays.

Splicing assays

For non-canonical splicing variants, use PS3 if there is RT-PCR and/or RNA sequencing

evidence demonstrating only abnormal splice products, with no evidence of normal splicing.

* Evidence of abnormal splicing includes transcripts of alternative length or with specific intron or exon inclusion/exclusion. These studies can be performed on mRNA extracted from patient-derived cells, or by inserting the mutant genomic DNA into plasmid vectors and introducing these into human or other mammalian host cells.

* Note that in patients who are compound heterozygotes for a splicing variant and another variants type that does not disrupt splicing, such as a missense variant, evidence of normal splicing is expected. However, the presence of normal splice products could complicate the assessment of the impact of the splice variant. Therefore, if there is any evidence of normal splice products, either when using RNA from patient cells or in an in vitro expression system, use PS3_Supporting.

* PP3 may also be used for non-canonical splice variants meeting PS3 or PS3_Supporting.

Modification Disease-specific

Type:

PS4

Original ACMG

Summary

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.

Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0.

See manuscript for detailed guidance.

Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Not Applicable

Comments:

The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. CCDS VCEP notes for PS4: This rule is typically used for autosomal dominant disorders, with PM3 used as a case-counting mechanism for autosomal recessive conditions.

PM1

Original ACMG

Summary

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Not Applicable

Comments:

Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation.

PM2**Original ACMG****Summary**

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting**Allele frequency <0.0004 (<0.04%) in all populations in gnomAD.**

CCDS VCEP notes: It is acceptable for a GAMT variant to be present in controls, if heterozygous, because GAMT-D is a recessive disorder. Homozygotes should not be seen in a population database, such as gnomAD, because the penetrance of this condition in individuals with biallelic pathogenic variants is expected to be 100%.

GAMT specifications:

- All subpopulations in gnomAD must have a maximum allele frequency less than 0.0004 (the highest population minor allele frequency of the most common pathogenic GAMT variant, c.327G>A, in gnomAD). Any variant with a frequency below this cutoff will meet PM2_Supporting (See Appendix 3). Note – PM2 will NOT be used at moderate strength; PM2 will only be applied as a Supporting criterion.
- If homozygotes are observed, the variant will meet BS2 (assuming 100% penetrance for an individual with 2 pathogenic variants in trans).

Modification Disease-specific, Strength

Type:

PM3**Original ACMG****Summary**

For recessive disorders, detected in trans with a pathogenic variant

Note: This requires testing of parents (or offspring) to determine phase.

Very Strong

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification Strength

Type:

Strong

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification Strength

Type:

Moderate

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification None

Type:

Supporting

- Follow SVI guidance for PM3 (https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf).
- Parental testing, or another appropriate molecular method (such as cloning each allele separately followed by sequencing), must have been performed in order to confirm that the variants are in trans if the patient is compound heterozygous.

Modification Strength

Type:

PM4

Original ACMG Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Moderate

Stop loss variants in GAMT have not been reported, as far as we are aware.

GAMT specifications: Use this rule “as is” for in frame deletions and insertions of 2 or more amino acids, but downgrade to PM4_Supporting for single amino acid deletions and insertions.

Modification None

Type:

Supporting

Downgrade to PM4_Supporting for single amino acid deletions and insertions.

Modification Strength

Type:

PM5

Original ACMG

Summary

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

Example: Arg156His is pathogenic; now you observe Arg156Cys.

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Moderate

If the pathogenicity of another missense change at the same amino acid residue is unknown, determine its pathogenicity using these specifications in order to determine if this criterion can be used. If the variant is pathogenic, use PM5. If the variant is likely pathogenic, use PM5_Supporting.

Modification None

Type:

Supporting

If the pathogenicity of another missense change at the same amino acid residue is unknown, determine its pathogenicity using these specifications in order to determine if this criterion can be used. If the variant is pathogenic, use PM5. If the variant is likely pathogenic, use PM5_Supporting.

Modification Strength

Type:

PM6

Original ACMG

Summary

Assumed de novo, but without confirmation of paternity and maternity.

Not Applicable

Comments:

Assumed de novo but without confirmation of paternity and maternity. CCDS VCEP notes for PS2 and PM6: De novo variants have not been reported in patients with GAMT deficiency, to our knowledge. Furthermore, the observation that a variant in GAMT has arisen de novo does not support its causality because GAMT deficiency is an autosomal recessive disorder. The occurrence of de novo variants is more supportive in autosomal dominant and X-linked disorders. Any de novo variants in GAMT, should they be observed, will be assessed based on the variant type, functional evidence, and in trans data as described.

PP1

**Original ACMG
Summary**

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.

Note: May be used as stronger evidence with increasing segregation data.

Not Applicable

Comments:

Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease. CCDS VCEP notes for PP1: Sibships large enough to use meet this criterion are extremely rare. In addition, because GAMT is the only gene involved in GAMT-D, ALL patients are expected to be bi-allelic, regardless of whether the pathogenic variants can be, or have been, detected. A variant under assessment may not be the true pathogenic variant but instead in linkage disequilibrium with an unidentified pathogenic variant. For this reason, this criterion does not facilitate assessment of pathogenicity.

PP2

**Original ACMG
Summary**

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

Not Applicable

Comments:

Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease. CCDS VCEP notes for PP2: Does not apply; there are benign and pathogenic missense variants in GAMT

PP3

Original ACMG

Summary

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).

Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Moderate

Non-canonical splice site variant predicted to be more deleterious, by SpliceAI and varSEAK, than a previously observed pathogenic variant at the same nucleotide.

Modification Strength

Type:

Supporting

- For missense changes, those with a REVEL score more than 0.75 will meet PP3.
- For in frame insertions and deletions, use PROVEAN and Mutation Taster. Results must be consistent to count.
- For non-canonical splice site variants (e.g., +3, -3), use SpliceAI (<https://spliceailookup.broadinstitute.org/>) and varSEAK (<https://varseak.bio/>). Results must be consistent to apply this criterion.
- For SpliceAI, any donor loss or acceptor loss with a score >0.5. For varSEAK, any variant with splicing class 4 or 5. Evidence for creation of a cryptic splice site should also be assessed.
- Do not apply this rule for canonical splice site changes meeting PVS1.

Modification None

Type:

PP4

Original ACMG

Summary

Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

Strong

4 points based on any combination of the following. Two or more data types are required to apply PP4_Strong:

- Elevated urine guanidinoacetate with or without low or low normal creatine (1 point)
- Elevated plasma guanidinoacetate with or without low or low normal creatine (2 points)
- Significantly decreased creatine peak in brain magnetic resonance spectroscopy with or without visible guanidinoacetate peak (3 points)
- GAMT enzyme activity <5% of normal (3 points)

* Variant must meet PM2_Supporting for PP4 to apply at any strength.

* For PP4 to be applied at strong, full GAMT gene sequencing, including all coding exons and intron/exon boundaries, must have been carried out. If not, consider downgrading.

Modification Disease-specific

Type:

Moderate

3 points based on any combination of the following. Two or more data types are recommended to apply PP4_Moderate:

- Elevated urine guanidinoacetate with or without low or low normal creatine (1 point)
- Elevated plasma guanidinoacetate with or without low or low normal creatine (2 points)
- Significantly decreased creatine peak in brain magnetic resonance spectroscopy with or without visible guanidinoacetate peak (3 points)
- GAMT enzyme activity <5% of normal (3 points)

* Variant must meet PM2_Supporting for PP4 to apply at any strength.

Modification Strength

Type:

Supporting

1-2 points based on:

- Elevated urine guanidinoacetate with or without low or low normal creatine (1 point)
- Elevated plasma guanidinoacetate with or without low or low normal creatine (2 points)

* Variant must meet PM2_Supporting for PP4 to apply at any strength

Modification Disease-specific

Type:

PP5

Original ACMG

Summary

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. [PubMed : 29543229](#) 

BA1

Original ACMG

Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Stand Alone

Allele frequency >0.003 (0.3%) in gnomAD in any continental population in gnomAD with >2000 alleles.

GAMT specifications:

- Any variant with a frequency >0.003 (based on the estimated prevalence 1 in 114,000, PMID 24071436) in gnomAD (max allelic contribution = 100%; max genetic contribution = 100%).
- Use the highest population minor allele frequency (MAF) in any given continental population with $>2,000$ alleles (European non-Finnish, African, East Asian, South Asian, Latino) (PMID 30311383).

Modification Disease-specific

Type:

BS1

Original ACMG

Summary

Allele frequency is greater than expected for disorder.

Strong

Allele frequency >0.001 (0.1%) in gnomAD in any continental population in gnomAD with >2000 alleles.

GAMT specifications:

- Any variant with a frequency >0.001 (based on the estimated prevalence 1 in 114,000 (PMID: 24071436) in gnomAD (max allelic contribution = 40%; max genetic contribution = 100%).
- Use the highest population minor allele frequency (MAF) in any given continental population with $>2,000$ alleles (European non-Finnish, African, East Asian, South Asian, Latino) (PMID 30311383).

Modification Disease-specific

Type:

BS2

Original ACMG

Summary

Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.

Strong

Observed in the homozygous state in a healthy adult.

Modification None

Type:

BS3

Original ACMG

Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Supporting

>30% normal GAA activity when the variant is expressed in a heterologous cell type.

GAMT specifications:

In vitro assays in which a variant is expressed in GAMT-deficient cultured cells (e.g. GAMT-deficient fibroblasts) or in-fusion High-Fidelity cloning of GAMT transcript and site directed mutagenesis to generate missense variant overexpressed in HeLa cells and measurement of GAMT activity in cells for wild-type and missense variant. Any variant with enzyme activity at or above 30% of normal in the following publications meets BS3_Supporting (Mercimek-Mahmutoglu et al, 2014; PMID 24415674; Mercimek-Mahmutoglu et al, 2016, PMID 26319512; DesRoches et al, 2016, PMID 26003046).

Modification Disease-specific,Strength

Type:

BS4

Original ACMG

Summary

Lack of segregation in affected members of a family.

Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Not Applicable

Comments:

Lack of segregation in a family. Caveat: The presence of phenocopies for common phenotypes.

BP1

Original ACMG

Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Not Applicable

Comments:

Does not apply. All types of variants cause GAMT-D.

BP2

Original ACMG

Summary

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

Supporting

Observed in cis with a pathogenic variant (to take AR inheritance into account).

Modification None

Type:

BP3

Original ACMG

Summary

In frame-deletions/insertions in a repetitive region without a known function.

Not Applicable

Comments:

There are no known repetitive regions without known function in GAMT.

BP4

Original ACMG

Summary

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Supporting

- For missense changes, REVEL score <0.5 .
- For in frame insertions and deletions, use PROVEAN and Mutation Taster. Results must be consistent to count.
- For non-canonical splice site variants, use SpliceAI (<https://spliceailookup.broadinstitute.org/>) and varSEAK (<https://varseak.bio/>) to assess the impact of variants that are not ± 1 or 2 canonical splice site variants. For SpliceAI, this criterion can be applied for scores <0.2 , and for varSEAK class 1 and 2.

If there is any evidence for possible creation of a cryptic splice site, this criterion should not be applied.

Modification None

Type:

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Not Applicable

Comments: An individual could be a carrier of a pathogenic variant in GAMT and have another disorder.

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee.

PubMed : 29543229 [↗](#)

BP7

Original ACMG Summary

A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Supporting

Apply this criterion as described.

Modification None

Type:

Rules for Combining Criteria

Pathogenic

1 Very Strong (*PVS1, PM3_Very Strong*) **AND** **≥ 1 Strong** (*PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong*)

1 Very Strong (PVS1, PM3_Very Strong) **AND** **≥ 2 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)

1 Very Strong (PVS1, PM3_Very Strong) **AND 1 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate) **AND 1 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

1 Very Strong (PVS1, PM3_Very Strong) **AND ≥ 2 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

≥ 2 Strong (PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong)

1 Strong (PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong) **AND ≥ 3 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)

1 Strong (PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong) **AND 2 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate) **AND ≥ 2 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

1 Strong (PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong) **AND 1 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate) **AND ≥ 4 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

Likely Pathogenic

1 Very Strong (PVS1, PM3_Very Strong) **AND 1 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)

1 Very Strong (PVS1, PM3_Very Strong) **AND ≥ 2 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

1 Strong (PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong) **AND 1 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)

1 Strong (PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong) **AND ≥ 2 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

≥ 3 Moderate (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)

2 Moderate (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate) **AND ≥ 2 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

1 Moderate (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate) **AND ≥ 4 Supporting** (PS3_Supporting, PM2_Supporting, PM3_Supporting, PM4_Supporting, PM5_Supporting, PP3, PP4)

1 Strong (PVS1_Strong, PS1, PS3, PM3_Strong, PP4_Strong) **AND 2 Moderate** (PVS1_Moderate, PM3, PM4, PM5, PP3_Moderate, PP4_Moderate)

Benign

≥ 2 Strong (BS1, BS2)

1 Stand Alone (BA1)

Likely Benign

1 Strong (BS1, BS2) **AND 1 Supporting** (BS3_Supporting, BP2, BP4, BP7)

≥ 2 Supporting (BS3_Supporting, BP2, BP4, BP7)